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Research article

Elevated CO_2 reduces the adverse effects of drought stress on a high-yielding soybean (*Glycine max* (L.) Merr.) cultivar by increasing water use efficiency

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ABSTRACT

Soybean (*Glycine max* (L.) Merr.) is the world's most important grain legume. The impacts of climate change such as elevated CO_2 and drought on soybean physiological and morphological responses are not well understood. This study evaluated the effects of elevated CO_2 (ambient concentration + 200 mmol mol^{-1}) and drought stress (35–45% of relative water content) on soybean leaf photosynthesis, chlorophyll fluorescence, stress physiological indexes, morphological parameters, biomass and yield over 2 years at the open-top chamber (OTC) experimental facility in North China. We found that drought decreased intrinsic efficiency of PSII (F_v'/F_m'), effective quantum yield of PSII photochemistry (Φ_{PSII}), photochemical quenching coefficient (qP), and yield of soybean, increased nonphotochemical quenching (NPQ), peroxidase (POD), and malondialdehyde (MDA), but had no effect on superoxide dismutase (SOD) or soluble sugar content. Elevated [CO_2] increased net photosynthetic rate (P_N), water-use efficiency (WUE), Φ_{PSII}, qP, SOD, soluble sugar content and yield of soybean. Elevated [CO_2] enhanced the positive effects of drought on WUE, but reduced the negative effects of drought on Φ_{PSII} and qP. Elevated [CO_2] enhanced the resistance to drought by improving the capacity of photosynthesis and WUE in soybean leaves.

1. Introduction

Rising atmospheric CO_2 concentration ([CO_2]), increasing temperature, and shifting precipitation patterns (more droughts) are predicted to have profound impacts on agricultural production (IPCC, 2013; Leakey et al., 2009; Shao et al., 2015). Elevated [CO_2] generally increases crop growth and yield (Gao et al., 2015; Han et al., 2015; Morgan et al., 2005), whereas drought may cause severe reduction in crop yield when it is beyond the ability of crop to acclimatize or recover (Bragazza, 2008; Jentsch et al., 2011). It has been reported that elevated [CO_2] can increase the photosynthetic rates and water-use efficiency (WUE) in some plants under drought stress, include C_3 (Qiao et al., 2010) and C_4 plants (Joseph and Leon, 2009; Leakey et al., 2006; Allen et al., 2011). This may compensate the drought-induced reduction in crop growth and yield (Ward et al., 2001; Lawlor and Cornic, 2002; Zinta et al., 2014).

Soybean (*Glycine max* (L.) Merr.) is the major grain legume and a significant source of protein for human consumption and livestock forage. The annual yield loss of soybean caused by drought is enormous (Sinclair et al., 2007; Sincik et al., 2008). Numerous studies on the impacts of elevated [CO_2] on soybean have shown that elevated [CO_2] may enhance the photosynthesis, WUE (by reducing stomatal conductance) and yield of soybean (Hao et al., 2012; Rogers et al., 2004; Morgan et al., 2005). The increased WUE under elevated [CO_2] implies that soybean may be tolerant to the drought conditions under future higher CO_2 conditions. Nonetheless, Li et al. (2013) showed that elevated [CO_2] improved the WUE and growth of soybean more effectively under normal water conditions than under drought stress. This indicates that elevated [CO_2] may not counteract the drought-induced reduction in soybean seed yield.

The antioxidative ability, soluble sugars and chlorophyll fluorescence have been used as indicators to evaluate drought stress in plants (Razavi et al., 2008; Hatata et al., 2013; Rosa et al., 2009). It was found that drought stress decreased effective quantum yield of PSII photochemistry (Φ_{PSII}) in strawberry (Razavi et al., 2008) and SOD in wheat (Hatata et al., 2013), but increased POD in wheat (Hatata et al., 2013)

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Abbreviations

$[CO_2]$	atmospheric CO_2 concentration
E	transpiration rate
EC	elevated atmospheric CO_2 concentration
ETR	electron transport rate
F_0	minimal fluorescence yield of the dark-adapted state
F_0'	minimal fluorescence yield of the light-adapted state
F_m	maximal fluorescence yield of the dark-adapted state
F_m'	maximal fluorescence yield of the light-adapted state
FM	fresh mass
F_s	the steady-state fluorescence yield
F_v/F_m	maximal quantum yield of PSII photochemistry
F_v'/F_m'	intrinsic efficiency of PSII
g_s	stomatal conductance
MDA	malondialdehyde
NPQ	nonphotochemical quenching
P_N	net photosynthetic rate
POD	peroxidase
qP	photochemical quenching coefficient
SOD	superoxide dismutase
WUE	water-use efficiency ($=P_N/E$)
Φ_{PSII}	effective quantum yield of PSII photochemistry

and soluble sugar concentrations in many plants (Rosa et al., 2009). However, no study has reported the drought effects on antioxidative ability, soluble sugars or chlorophyll fluorescence in soybean under elevated $[CO_2]$. The information is important for understanding the mechanisms for the response to drought of soybean grown under elevated $[CO_2]$. Here we investigate the effects of elevated $[CO_2]$ and drought on the photosynthesis, antioxidative ability, chlorophyll fluorescence, soluble sugars and WUE of soybean, and their relation to the growth and yield response of soybean to elevated $[CO_2]$. We aimed to address following questions: (i) can elevated $[CO_2]$ soybean from photoinhibition and water loss by drought?; and (ii) is elevated $[CO_2]$-enhanced drought tolerance related to antioxidative ability and/or changes in soluble sugars levels? Answering these questions will provide insights into soybean crop management under climate change.

2. Materials and methods

2.1. Experimental design

The experiment was carried out using the OTC facility at Shanxi Agricultural University (37.42°N and 112.55°E), Taigu, Shanxi, China. The $[CO_2]$ of control OTC (CK) was maintained at the ambient $[CO_2]$ and the elevated $[CO_2]$ OTC (EC) at $[CO_2]$ of ambient + 200 mmol mol^{-1} from crop emergence to harvest. The facility operational procedures were described in Hao et al. (2017). A high-yielding soybean cultivar [Zhonghuang 35, bred by the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences] was sown in pots (size of one plot: 60 cm × 40 cm × 28 cm, length × width × height) on 13 June 2013 and 16 June 2014. Eight plants were planted in each pot and there were ten replicate pots in each chamber. Two water treatments were applied 25 days after sowing (the branching stage): (i) soil water content were 60–80% of relative water content (RWC), i.e. no drought; and (ii) 35–45% of RWC, i.e. drought. The water content was measured by wet sensor (KZSF, China) and maintained at the targeted moisture regimes by irrigation during the growth period. Fertilizers were applied at the rates of 11.04 g N pot^{-1} and 12.24 g P pot^{-1} during the elongation stage. Plants were exposed to sunlight and temperatures were maintained at an average of 23.1 °C and 22.2 °C in CK OTC in 2013 and 2014, respectively. The corresponding values in EC OTC were 22.8 °C and 22.0 °C in 2013 and 2014. Relative humidity was maintained at 60–70% throughout the soybean growing season.

2.2. Gas exchange measurements

Leaf gas exchange measurements were made at the full-bloom stage (53 days after sowing in 2013 and 51 days after sowing in 2014) and seed filling stage (80 days after sowing in 2013 and 82 days after sowing in 2014). The upper most fully-expanded leaves was used for measuring gas exchange by a portable gas exchange system (LI-COR 6400; LI-COR, Lincoln, Neb, USA). The measurements were performed from 09:00 to 11:30 a.m. with 1400 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) after 3 min of light adaptation. The leaf chamber temperature was set at approximately 28 °C. The vapour pressure deficit (VPD) on the leaf surface ranged from 1.9 to 2.1 kPa. P_N, stomatal conductance (g_s), and transpiration rate (E) were measured at the same irradiance, temperature and vapour pressure. WUE was calculated as P_N/E). The $[CO_2]$ in the leaf chamber was maintained 400 μmol mol^{-1} for CK OTC, and 600 μmol mol^{-1} for EC OTC.

2.3. Chlorophyll fluorescence

On the same day with the gas exchange measurements, chlorophyll fluorescence emission from the upper most fully-expanded flag leaf surface was measured by using a miniaturized pulse-amplitude modulated fluorescence analyzer (PAM-2100, Walz, Effeltrich, Germany). Minimal fluorescence yield of the light-adapted state (F_0'), maximal fluorescence yield of the light-adapted state (F_m') and the steady-state fluorescence yield (F_s) was measured with a PPFD of 4000 μmol (photon) $m^{-2}s^{-1}$ and a duration of 800 ms between 08:30 and 11:30 a.m. Minimal fluorescence yield of the dark-adapted state (F_0) and maximal fluorescence yield of the dark-adapted state (F_m) of darkness-adapted leaves were investigated between 23:00 on the same day and 01:00 h on the next day. Other chlorophyll fluorescence parameters including the effective quantum yield of PSII photochemistry ($\Phi_{PSII}=(F_m'-F_s)/F_m'$), maximal quantum yield of PSII photochemistry ($F_v/F_m=(F_m-F_0)/F_m$), intrinsic efficiency of PSII ($F_v'/F_m'=(F_m'-F_0')/F_m'$) and non-photochemical quenching ($NPQ=(F_m-F_m')/F_m'$), were determined as described by Kramer et al. (2004). Photochemical quenching coefficient ($qP=(F_m'-F_s)/(F_m'-F_0')$) was calculated as described by Krause and Weis (1991).

2.4. Determination of malondialdehyde (MDA), peroxidase (POD), superoxide dismutase (SOD), and soluble sugar concentrations

All of the upper most fully-expanded flag leaves of four pots (out of the 10 replicate pots) were taken for analysis of the MDA, POD, SOD and soluble sugar concentrations at filling seed stage (81 days after sowing) in 2013 using the methods below.

MDA content was determined by the thiobarbituric acid (TBA) test following the protocol given by Heath and Packer (1968). About 0.5 g leaf segments were homogenized in 5.0 ml of 5% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 g for 10 min. The supernatant was assayed for spectrophotometric MDA concentration. All spectrophotometric analyses were performed using a 722S spectrophotometer (INESA, Shanghai, China).

POD activity was assayed as enzyme units per gram fresh weight (U/g fw) according to Sakharov and Aridilla (1999), which depends on the increase in absorbance at 470 nm. The assay mixture contained 0.1 ml enzyme extract, 0.1 ml H_2O_2 (2%) and 2.8 ml of guaiacol (3%). Absorbance change of 1.0permin was defined as one unit of POD activity.

SOD activity was assayed at 560 nm according to the method of

Sgherri et al. (1994), which depends on inhibiting the photochemical reduction of nitro blue tetrazolium (NBT). One unit of SOD represented the amount of enzyme for a 50% inhibition of the photo-reduction of NBT.

Soluble sugar content was assayed following the anthrone colorimetric method (Luo and Huang, 2011). The 0.3 g fresh leaf sample was used. Then the absorbency at 620 nm wavelength was measured The total soluble sugar concentration was calculated according to Luo and Huang (2011).

2.5. Harvesting

At maturity, soybean plants (the six replicate pots that were not used for the analysis of the concentrations of MDA, POD, SOD and soluble sugar) were harvested on 6 October 2013 (117 days after sowing) and 8 October 2014 (116 days after sowing). Samples was air-dried until constant weight in a drying oven. After drying, the height, stem diameter and node number were determined for five plants of each pot. Then all plants were separated into leaves, stems, pods and seeds, and weighed. The mass, the number of pods and the number of seed were recorded to calculate the number of seeds per pod, the mass of 100 seeds and yield.

2.6. Statistical analysis

A split-plot design was employed with CO_2 concentration as the whole-plot treatment and drought as the split-plot treatment. An analysis of variance (ANOVA) with four-way ANOVA by SAS System 8.1 (SAS Institute Inc., USA) was used to test the effects of CO_2 concentration, drought, year and stage on P_N, gas exchange parameters, and chlorophyll fluorescence of soybean. Three-way ANOVA was used to test whether CO_2 concentration, drought or year, alone or in interaction, had a significant influence on the above-ground biomass, yield, plant morphological parameters, weight and yield component of soybean. Two-way ANOVA was used to test the effects of CO_2 concentration and drought on POD, SOD, MDA and soluble sugar content of soybean. Treatments were compared by Duncan's multiple range tests at $P = 0.05$.

3. Results

3.1. P_N and gas exchange parameters

Elevated [CO_2] significantly increased P_N (by 44.1%) and WUE (by 115.6%), decreased E by 16.5% in both 2013 and 2014, but did not affect g_s in either year. Under drought condition, the elevated [CO_2]-induced increase in P_N was markedly greater at the full-bloom stage (99.5%) than at the seed filling stage (1.5%). Drought decreased P_N (by 44.3%), g_s (by 61.2%), C_i (by 8.3%) and E (by 47.2%), but increased WUE (by 49.2%) in both years. The interaction between CO_2 and drought on WUE was significant ($P < 0.01$), but not for P_N, g_s or E. Elevated [CO_2] enhanced the positive effects of drought on WUE. Specifically, the increase in WUE by EC × Drought interaction (5.78 = 9.35-3.56) was significantly greater than that by Drought (0.33 = 3.89-3.56) plus EC (1.74 = 5.31-3.56) (Table 1).

3.2. Chlorophyll fluorescence

Maximal quantum yield of PSII photochemistry (F_v/F_m), intrinsic efficiency of PSII (F_v'/F_m') and non-photochemical quenching (NPQ) was not significantly affected by elevated [CO_2] in either year. Elevated [CO_2] significantly increased effective quantum yield of PSII photochemistry (Φ_{PSII}) (by 17.1%) and photochemical quenching coefficient (qP) (by 27.0%) in both years. Under drought condition, elevated [CO_2] increased Φ_{PSII} (by 16.0%) and qP (by 16.8%) in both years. Drought decreased F_v/F_m (by 1.1%), F_v'/F_m' (by 17.7%), Φ_{PSII} (by 20.5%) and qP (by 10.1%) in both years, but increased NPQ by 44.8%. CO_2 and drought had no interactive effects on F_v/F_m, F_v'/F_m' or NPQ, but the

Table 1
Effects of elevated [CO_2] and drought on gas exchange parameters in the upper most fully-expanded leaves of soybean. Values are means of 10 replicates. Mean values with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Year	Stage	Water conditions	Growth [CO_2]	P_N [molm^{-2} s^{-1}]	g_s [mol (H_2O) m^{-2} s^{-1}]	E [mmol (H_2O) m^{-2} s^{-1}]	WUE [mol (CO_2)mol (H_2O)$^{-1}$]
2013	full-bloom stage	Normal	CK	13.84 c	0.24 bc	3.18 b	4.48 d
			EC	17.55 b	0.20 c	2.97 b	5.94 c
		Drought	CK	6.91 de	0.07 d	1.62 c	4.77 cd
			EC	10.63 c	0.08 d	1.47 c	7.22 b
	seed filling stage	Normal	CK	7.58 de	0.11 d	2.96 b	2.67 fg
			EC	10.31 c	0.08 d	2.15 bc	4.81 d
		Drought	CK	11.00 c	0.20 c	4.77 a	2.33 g
			EC	10.18 cd	0.08 d	2.18 bc	4.51 d
2014	full-bloom stage	Normal	CK	17.46 b	0.32 ab	4.62 a	3.79 e
			EC	24.19 a	0.41 a	5.00 a	4.85 d
		Drought	CK	7.55 de	0.08 d	1.57 c	4.92 cd
			EC	17.21 b	0.17 cd	2.83 b	6.29 c
	seed filling stage	Normal	CK	17.31 b	0.34 ab	5.29 a	3.32 ef
			EC	25.34 a	0.31 ab	4.65 a	5.63 c
		Drought	CK	4.93 e	0.07 d	1.52 c	3.55 ef
			EC	5.97 de	0.01 e	0.32 d	19.36 a
P value	year			0.00	0.00	0.00	0.00
	stage			0.00	0.00	0.66	0.07
	CO_2			0.00	0.42	0.00	0.00
	drought			0.00	0.00	0.00	0.00
	year * stage			0.48	0.30	0.00	0.00
	year * CO_2			0.00	0.02	0.01	0.00
	year * drought			0.00	0.00	0.00	0.00
	stage * CO_2			0.00	0.00	0.00	0.00
	stage * drought			0.59	0.02	0.13	0.00
	CO_2* drought			0.08	0.58	0.30	0.00
	year * stage * CO_2			0.67	0.17	0.74	0.00
	year * stage * drought			0.00	0.00	0.00	0.00
	stage * CO_2 * drought			0.00	0.15	0.02	0.00
	year * stage * CO_2 * drought			0.33	0.73	0.30	0.00

interaction between CO_2 and drought on Φ_{PSII} ($P < 0.05$) and qP ($P < 0.01$) was significant. Elevated [CO_2] reduced the negative effects of drought on Φ_{PSII} and qP (Table 2).

3.3. Determination of POD, SOD, MDA and soluble sugar concentrations

Elevated [CO_2] had no effect on POD or MDA, but increased SOD by 13.5% and soluble sugar content by 26.9% in soybean leaves. Drought did not affect SOD or soluble sugar content, but increased POD by 22.4% and MDA by 23.8%. There was no significant interaction between CO_2 and drought on POD, SOD, MDA or soluble sugar content (Table 3).

3.4. Plant morphological parameters

Elevated [CO_2] significantly increased node number (by 8.5%) and stem diameter (by 22.6%) in both years, but had no effect on the height of soybean. Drought decreased height (by 32.4%), node number (by 25.0%) and stem diameter (by 35.7%) in both years. CO_2 and drought had no interactive effects on height, node number or stem diameter (Table 4).

3.5. Biomass and yield

Elevated [CO_2] significantly increased the number of pod per plant by 16.6% for two years, but had no effect on seeds number per pod or the mass of 100 seeds. Drought decreased the number of pod per plant, seed number per pod and the mass of 100 seeds by 42.4, 25.2 and 23.9%, respectively (Table 4).

Elevated [CO_2] significantly increased the above-ground biomass (per m^2) by 17.5% averaged across two years, and the increase under drought (22.6%) was greater than that under no drought condition (15.2%) (Table 5). Elevated [CO_2] significantly increased the yield in both years by an average of 17.7%, and the increase under drought (29.6%) was greater than that under no drought (12.9%) (Table 5). Drought decreased the above-ground biomass and yield in both years by an average of 44.9% and 55.9%, respectively (Table 5).

4. Discussion

POD and SOD form the first line of defense against reactive oxygen species (ROS). Malondialdehyde (MDA) is a marker for oxidative stress. Changes in their activity and amounts in homoiohydric plants under drought stress have been used as an indicator of a redox status change (Moran et al., 1994; Schwanz and Polle, 2001). Soluble sugars, as nutrient and metabolite signaling molecules, are involved in the regulation of physiological processes of plant responses to a number of stresses (Couée et al., 2006; Rosa et al., 2009). We found that elevated [CO_2] increased SOD and soluble sugar in soybean leaves whereas drought increased POD and MDA. However CO_2 and drought had no interactive effects on POD, SOD, MDA or soluble sugar content (Table 3). This suggests that elevated [CO_2] could not alleviate drought-induced negative effects on the defense system of soybean against reactive oxygen species or osmotic adjustment.

As the substrates of photosynthesis, CO_2 and water are crucial to crop growth and yield (Li et al., 2013; Beardall and Raven, 2004). Elevated [CO_2] has the potential to increase crop WUE by enhancing photosynthesis and reducing leaf transpiration (Leakey et al., 2006; Gao et al., 2015). This may help crops to acclimatize the drought that is predicted to be more prevalent in the near future (Joseph and Leon, 2009; Lawlor and Cornic, 2002). We found that elevated [CO_2] enhanced the positive impacts of drought on WUE of soybean. Chlorophyll fluorescence is a tool for evaluation of drought stress (Razavi et al., 2008). In our study, drought decreased F_v/F_m, F_v'/F_m', qP and Φ_{PSII} of soybean, but increased NPQ whereas elevated [CO_2] reduced drought-induced negative effects on Φ_{PSII} and qP. The above findings indicate that elevated [CO_2] improved the capacity of photosynthesis (like Φ_{PSII} and qP) and WUE of soybean and enhanced its resistance to drought

Table 2
Effects of elevated [CO_2] and drought on chlorophyll fluorescence parameters in the upper most fully-expanded leaves of soybeans. Values are means of 10 replicates. Mean values with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Year	Stage	Water conditions	Growth [CO_2]	Fv/Fm	Fv'/Fm'	Φ_{PSII}	qP	NPQ
2013	full-bloom stage	Normal	CK	0.80 abc	0.56 a	0.44 ab	0.78 bc	1.20 ef
			EC	0.82 a	0.59 a	0.49 a	0.82 bc	1.28 e
		Drought	CK	0.81 ab	0.43 b	0.39 b	0.75 c	1.96 c
			EC	0.81 ab	0.58 a	0.44 ab	0.93 ab	1.14 ef
	seed filling stage	Normal	CK	0.78 cd	0.48 b	0.32 c	0.65 cd	1.39 de
			EC	0.73 e	0.38 bc	0.39 b	1.14 a	1.28 def
		Drought	CK	0.78 cd	0.45 b	0.32 c	0.77 abc	1.60 d
			EC	0.78 cd	0.43 b	0.39 b	0.78 c	1.49 de
2014	full-bloom stage	Normal irrigation	CK	0.80 bc	0.53 ab	0.30 c	0.57 de	1.61 cde
			EC	0.82 a	0.58 a	0.42 ab	0.72 c	1.37 de
		Drought	CK	0.76 de	0.41 b	0.23 d	0.55 ef	3.37 a
			EC	0.80 abc	0.32 c	0.25 cd	0.64 cd	2.10 bc
	seed filling stage	Normal irrigation	CK	0.81 ab	0.59 a	0.28 cd	0.47 f	1.05 fg
			EC	0.81 ab	0.46 b	0.24 d	0.51 ef	1.74 c
		Drought	CK	0.78 cd	0.40 b	0.12 f	0.30 h	2.29 b
			EC	0.78 cd	0.42 b	0.15 e	0.34 g	1.86 c
P value	year			0.10	0.10	0.00	0.00	0.00
	stage			0.00	0.00	0.00	0.00	0.04
	CO_2			0.42	0.46	0.05	0.05	0.38
	drought			0.03	0.00	0.00	0.00	0.00
	year * stage			0.00	0.00	0.80	0.00	0.01
	year * CO_2			0.00	0.07	0.28	0.87	0.00
	year * drought			0.00	0.00	0.00	0.23	0.00
	stage * CO_2			0.00	0.00	0.03	0.33	0.34
	stage * drought			0.09	0.01	0.83	0.00	0.04
	CO_2* drought			0.12	0.06	0.04	0.00	0.66
	year * stage* CO_2			0.68	0.05	0.26	0.00	0.02
	year * stage* drought			0.15	0.85	0.86	0.81	0.15
	stage * CO_2* drought			0.02	0.02	0.68	0.09	0.02
	year * stage* CO_2* drought			0.06	0.00	0.05	0.03	0.00

Table 3
Effects of elevated [CO_2] and drought on POD, SOD, MDA and soluble sugar content in the fully-expanded flag leaf of soybeans in filling seed stage. Values are means of 4 replicates. Mean values with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Water conditions	Growth [CO_2]	POD (ug·g^{-1}FW·min^{-1})	SOD (u·g^{-1}FW·h^{-1})	MDA (mmol·g^{-1}FW)	Soluble sugar content (mg· g $^{-1}$FW)
Normal	CK	22.53 a	146.15 b	0.22 b	5.57 c
	EC	19.90 b	163.14 a	0.20 c	7.27 ab
Drought	CK	27.40 a	145.09 b	0.27 a	6.49 b
	EC	24.56 a	167.31 a	0.25 b	8.04 a
P value	CO_2	0.19	0.00	0.14	0.04
	drought	0.03	0.78	0.01	0.25
	CO_2* drought	0.95	0.63	0.88	0.91

Table 4
Effects of elevated [CO_2] and drought on plant morphological parameters, weight and yield component of soybean. Values are means of 6 replicates. Mean values with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Year	Water conditions	Growth [CO_2]	Height [cm]	Node number	Stem diameter [cm]	Number of pods per plant	Number of seeds per pod	mass of 100 seeds [g]
2013	Normal	CK	65.47 b	10.34 c	0.47 b	22.83 b	1.92 ab	13.89 ab
		EC	61.41 b	9.74 cd	0.51 ab	25.38 ab	2.14 ab	11.89 b
	Drought	CK	47.36 c	7.86 e	0.35 d	16.26 cd	1.71 ab	13.42 ab
		EC	46.44 c	8.58 de	0.40 c	17.29 c	1.60 ac	10.91 bc
2014	Normal	CK	67.21 b	12.43 b	0.42 c	23.49 ab	1.86 b	16.24 a
		EC	74.99 a	14.25 a	0.52 a	26.81 a	1.85 b	17.28 a
	Drought	CK	40.96 d	8.61 de	0.27 e	9.11 e	1.05 d	9.48 c
		EC	47.07 c	10.02 c	0.33 d	14.1 d	1.45 c	11.26 bc
P value	year		0.08	0.00	0.00	0.01	0.03	0.11
	CO_2		0.10	0.00	0.00	0.01	0.30	0.49
	drought		0.00	0.00	0.00	0.00	0.00	0.00
	year*CO_2		0.00	0.00	0.10	0.23	0.56	0.01
	year*drought		0.00	0.00	0.03	0.00	0.33	0.00
	CO_2*drought		0.78	0.38	0.56	0.81	0.87	0.93
	year*CO_2*drought		0.35	0.10	0.37	0.38	0.14	0.61

Table 5
Effects of elevated [CO_2] and drought on the above-ground biomass and yield of soybean. Values are means of 6 replicates. Mean values with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Year	Water conditions	Growth [CO_2]	Above-ground biomass [g m^{-2}]	Yield [g m^{-2}]
2013	Normal	CK	491.67 c	200.00 b
		EC	542.67 c	207.00 b
	Drought	CK	322.00 d	125.33 c
		EC	335.33 d	127.33 c
2014	Normal	CK	752.67 b	235.33 b
		EC	903.67 a	284.33 a
	Drought	CK	155.33 e	52.33 d
		EC	304.33 d	103.00 c
P value	year		0.00	0.57
	CO_2		0.01	0.05
	drought		0.00	0.00
	year*CO_2		0.02	0.02
	year*drought		0.00	0.00
	CO_2*drought		0.53	0.50
	year*CO_2*drought		0.56	0.45

(Tables 1 and 2). This is consistent with other studies on rice (Baker et al., 1997), sugarcane (Joseph and Leon, 2009) and sorghum (Ottman et al., 2001), but in contrast to a study on soybean (Li et al., 2013). Li et al. (2013) found that elevated [CO_2] improved the WUE and growth of soybean to a greater extent under normal water conditions than under drought stress, and did not affect soybean yield under drought stress. On the other hand, we found that elevated [CO_2] alleviated drought-induced negative effects on above-ground biomass and grain yield of this soybean cultivar, which was facilitated by the improved capacity of photosynthesis and WUE under drought and elevated [CO_2]. While the soybean cultivar we used was a high-yielding one and they used a high-oil cultivar, the contrasting results between our study and that of Li et al. (2013) might be attributed to the intraspecific variation of soybean response to elevated [CO_2], which has been reported for soybean growth, yield (Ziska et al., 1999), photosynthesis (Hao et al., 2012), nitrogen fixation capacity (Lam et al., 2012), and N, P and K uptake (Hao et al., 2016). Understanding the intraspecific variation of soybean physiological and morphological response to the interaction between elevated [CO_2] and drought is critical for breeding a high-yielding cultivar that will adapt well to future climates, which warrants further research.

5. Conclusion

Drought decreased P_N, g_s, C_i, E, F_v/F_m, F_v'/F_m', Φ_{PSII}, qP in soybean, but increased WUE, NPQ, POD, MDA, and had no effect on SOD or soluble sugar content. Elevated [CO_2] enhanced the positive effects of drought on WUE, but reduced the negative effects of drought on Φ_{PSII} and qP. This suggests that elevated [CO_2] enhanced the drought tolerance of soybean through improving its photosynthetic capacity and WUE under drought stress. This study demonstrated that elevated [CO_2] would benefit soybean production in the arid regions of northern China under the future drier climates. Our results provide implications for sustainable soybean production and breeding drought tolerant soybean cultivars for rainfed cropping systems under future higher CO_2 and potentially drier environments.

Author contribution

Xingyu Hao and Frank Yonghong Li designed experiments. Aiping Wang and Yuzheng Zong carried out experiments. Heran Wang analyzed experimental results. Xingyu Hao and Ping Li wrote the manuscript. Shu Kee Lam carried out writing-review and editing.

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